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Review

Prokaryotic toxin-antitoxin systems — the role in bacterial physiology and application in molecular biology

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Bacteria have developed multiple complex mechanisms ensuring an adequate response to environmental changes. In this context, bacterial cell division and growth are subject to strict control to ensure metabolic balance and cell survival. A plethora of studies cast light on toxin-antitoxin (TA) systems as metabolism regulators acting in response to environmental stress conditions. Many of those studies suggest direct relations between the TA systems and the pathogenic potential or antibiotic resistance of relevant bacteria. Other studies point out that TA systems play a significant role in ensuring stability of mobile genetic material. The evolutionary origin and relations between various TA systems are still a subject of a debate. The impact of toxin-antitoxin systems on bacteria physiology prompted their application in molecular biology as tools allowing cloning of some hard-to-maintain genes, plasmid maintenance and production of recombinant proteins.

Keywords: antibiotic resistance, bacteria physiology, environmental stress conditions, toxin-antitoxin systems

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INTRODUCTION

Toxin-antitoxin systems emerged in research in mid 80's. A detailed insight into their functions and mechanisms of action has been gained in the last two decades and brought several interesting conclusions as to the importance of such systems for bacterial physiology. The term "toxin-antitoxin system", usually abbreviated as "TA system", comprises a functional element consisting, in most cases, of a biologically active protein molecule and a corresponding inhibitor, whose nature and inhibitory mechanism depend on the system's class affiliation. Components of such systems are encoded within policistronic operons, often with partially overlapping open reading frames. The systems are widespread among *Bacteria* as well as *Archaea* (Mittenhuber, 1999; Gerdes, 2000; Pandey & Gerdes, 2005; Makarova *et al.*, 2009) and evolved to carry out diverse functions. However, their common feature is an enzymatic activity detrimental for the cell metabolism. Such toxic activity has been demonstrated to switch bacterial cells over to a dormant state, leading to cell death during prolonged exposure. In most cases various stress stimuli are responsible for TA system activation. The signalling pathway in such instances is often related to other stress-induced response pathways. Moreover, it is well documented that in some cases the activity of TA systems stabilizes mobile genetics elements, therefore comprising an important mechanism of plasmids maintenance. In the light of the increasing multi-drug resistance among virulent strains, reports on the potential relation between TA systems and modulation of pathogen–host interactions seem to be of utmost importance.

CLASSIFICATION OF TOXIN-ANTITOXIN SYSTEMS

The biological activity of a toxin comprising a component of a TA systems is usually (but not always) that of an endoribonuclease. Bioinformatic analysis of multiple available sequences of bacterial genetic elements points to multiple novel, putative TA *loci* and suggests that many of known TA systems, bacterial as well as archaeal, are evolutionarily related (Anantharaman & Aravind, 2003; Hayes & Sauer, 2003; Gerdes *et al.*, 2005; Sevin & Barloy-Hubler, 2007; Makarova *et al.*, 2009; Weaver *et al.*, 2009; Arbing *et al.*, 2010). The classification of TA systems is based on the mechanism of inhibition of the toxin as well as on operon autoregulatory functions. Initially two classes of TA systems were identified (Gerdes & Wagner, 2007), but subsequent discoveries extended the classification to three classes (Blower *et al.*, 2009). Recent studies suggest the existence of yet another type, namely a three-component TA system (Hallez *et al.*, 2010). As immediately visible from the above discussion the field is in a constant and dynamic growth and one may expect that many interesting findings are likely to emerge in the following years.

Class I includes systems in which the antitoxin is an antisense RNA forming duplexes with the toxin mRNA. This leads to inhibition of translation in a process known as RNA interference. Examples of such systems are chromosomally located operons found in *Escherichia coli*, namely *tisAB* (Vogel *et al.*, 2004) and *symER* (Kawano *et al.*, 2007), as well as plasmid loci *parB* (Gerdes *et al.*, 1986) of *E. coli* and *par* of *Enterococcus faecalis* (Greenfield *et al.*, 2000; Weaver *et al.*, 2009) and a homologous plasmid operon of *Staphylococcus aureus* (Jensen *et al.*, 2010). Among the mentioned systems toxins have multiple different roles. For example the SymE toxin is an mRNA interferase encoded in the *symER* operon. The toxin binds ribosomes to exert its activity (Kawano *et al.*, 2007). The TisB toxin, which is encoded in the *tisAB* operon (Vogel *et al.*, 2004) decreases the protonmotoric force across the bacterial cell membrane and cause subsequent drop in ATP production, which leads

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Abbreviations: TA system, toxin-antitoxin system; ppGpp, 3′,5′-guanosine bisphosphate; NMR, nuclear magnetic resonance; SPP system, single protein production system

to metabolic dormancy (Unoson & Wagner, 2008). Hok toxin, encoded in the *parB* operon, irreversibly damages the cell membrane (Gerdes *et al.*, 1986). In the latter case the regulation of the toxin level is indirect. RNA interference suppresses expression of the gene *mok*, which is a regulator of *hok* gene transcription (Thisted & Gerdes, 1992).

Class II encompasses a wide range of TA systems. Antitoxins of this class are proteins. The biological activities exhibited by the toxins include transcription inhibition by targeting gyrase function and interference with translation through an mRNA interferase activity, which may or may not rely on ribosome binding. The endoribonucleolytic activity of mRNA interferases is often sequence specific. Table 1 gives a short overview of the class II TA systems and their characteristics.

Class III comprises a single member only. This system is encoded in the *toxIN* operon of *Erwinia carotonovora*, a plant pathogen. In this case inhibition of ToxN toxin activity is driven by RNA molecules directly interacting with the toxin molecules (Blower *et al.*, 2009; Fineran *et al.*, 2009).

RELATIONS AND STRUCTURAL SIMILARITIES AMONG CLASS II TA SYSTEMS

The evolutionary relationship among class II TA systems is a subject of an open debate. Attention is mainly focused on toxins since there is a substantial sequence and structural variety among the antitoxins. Ten TA families of class II have been described so far (Pandey & Gerdes, 2005; Jorgensen *et al.*, 2009; Van Melderen & Saavedra De Bast, 2009) and for three of them, *relBE*, *parDE* and *higBA*, a phylogenetic relationship based on sequence similarities has been proposed (Anantharaman & Aravind, 2003; Tsilibaris *et al.*, 2007). Strikingly, the toxin of the *parDE* system is a gyrase inhibitor in contrast to the toxins of the *relBE* and higBA systems, which are mRNA interferases. A broader analysis of this issue leads to other interesting conclusions. There is no evidence for an evolutionary relation between the *ccdAB* and *parDE* systems (Anantharaman & Aravind, 2003) although the toxin of the *ccdAB* system is also a gyrase inhibitor. However, there is a significant structural similarity between the toxins of the *ccdAB* and *kis/kid (parD)* systems (Diago-Navarro *et al.*, 2010), which, similarly to the *parDE* and *relBE* or *higBA* systems, are a gyrase inhibitor and an mRNA interferase, respectively. Other reports point to a structural similarity among the toxins of the *ygiUT (mqsRA)*, *relBE* and *yefM-yoeB* systems as well as RNase Sa of *Streptomyces aureofaciens* (Brown *et al.*, 2009).

Not only among RelE homologues is a similarity with RNase Sa noticeable. Toxins of the *ccdAB* and *kis/kid* or *mazEF (chpAK)* systems are also structurally similar. This similarity is related to the presence of a β-sheet core in these molecules (Fig. 1). However, this β-sheet core structure is most likely related to the ability to form dimers (Miller, 1989) rather than reflects evolutionary or functional relationships. Structural analysis of mRNA interferases and comparative studies allow the deduction of the mechanism of their endoribonucleolytic activiy (Agarwal *et al.*, 2009; Brown *et al.*, 2009; Diago-Navarro *et al.*, 2010). Tracing evolutionary relations among the TA systems is difficult because of the fast specialisation of TA system components (Arbing *et al.*, 2010). It has been reported that the toxin of the *phd/doc* system is similar to a virulence factor toxic to eukaryotic host cells (Arbing *et al.*, 2010). Another example is the sequence similarity of toxins of the *symER* and *phd/doc* systems to antitoxins of other TA systems — *yefM-yoeB* (Arbing *et al.*, 2010) and *mazEF* (Kawano *et al.*, 2007), respectively.

REGULATION OF CLASS II TA SYSTEM ACTIVITY

In operons of class II TA systems an antitoxin gene is usually, but not always, located upstream a gene for a toxin. The order is reversed for example in the *higBA*,

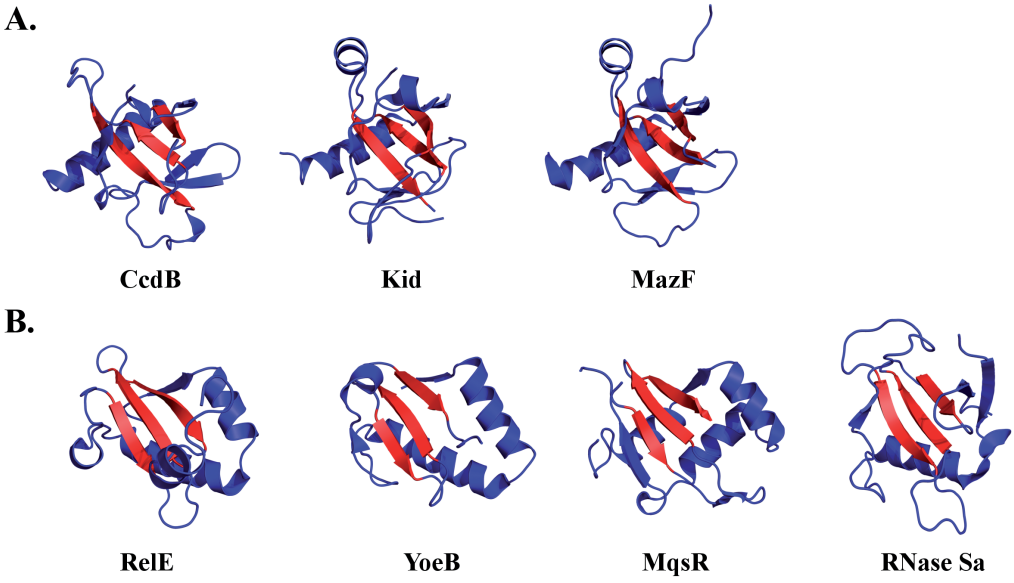

Figure 1. Structural similarities among toxins belonging to different families
(A) ccdBA and mazEF (Diago-Navarro *et al.*, 2010); **(B)** relBE and RNase Sa of *Streptomyces aureofaciens* (Brown *et al.*, 2009). In fact, β-sheet core (red) structure is similar among all these toxins. Models prepared with PyMOL ver. 1.1r2pre (DeLano WL, 2002). Structures' PDB IDs — CcdB: 1VUB; Kid: 1M1F; MazF: 1UB4; RelE: 2KC8; YoeB: 2A6Q; MqsR: 3HI2; RNase Sa: 1RSN.

Table 1. Ten families of class II TA systems and data about well-researched members

Family	Operon	Toxin	Antitoxin	Source organism/location	Activity	Mechanism of toxicity
ccdAB	ccdAB	CcdB	CcdA	*Escherichia coli*/plasmid[1]	gyrase inhibitor[2]	transcription inhibition[2]
parDE	parDE	ParE	ParD	*Escherichia coli*/plasmid[3]	gyrase inhibitor[4]	transcription inhibition[4]
phd/doc	phd/doc	Phd	Doc	prophage P1[5]	binding ribosome 30S subunit[6]	translation inhibition[6]
mazEF	mazEF (chpAK)	MazF (ChpK)	MazE (ChpA)	*Escherichia coli*/chromosome[7]	endoribonuclease[8]	translation inhibition[8]
	kis/kid (parD)	Kid	Kis	*Escherichia coli*/plasmid[9]	endoribonuclease[10]	translation inhibition[10]
	pemIK	PemK	PemI	*Escherichia coli*/plasmid[11]	endoribonuclease[12]	translation inhibition[12]
	chpBIK	ChpBK	ChpBI	*Escherichia coli*/chromosome[13]	endoribonuclease[14]	translation inhibition[14]
	mazEF-mt1 – mazEF-mt7	MazF-mt1 – MazF-mt7	MazE-mt1 – MazE-mt7	*Mycobacterium tuberculosis*/chromosome[15]	MazF-mt-1,3,6,7 – endoribonuclease, others not researched[15]	MazF-mt-1,3,6,7 – translation inhibition, others not researched[15]
	$mazEF_{Sa}$	$MazF_{Sa}$	$MazE_{Sa}$	*Staphylococcus aureus*/chromosome[16]	endoribonuclease[16]	translation inhibition[16]
	$pemIK_{Sa}$	$PemK_{Sa}$	$PemI_{Sa}$	*Staphylococcus aureus*/plasmid[17]	endoribonuclease[18]	unknown
relBE	relBE	RelE	RelB	*Escherichia coli*/chromosome[19]	endoribonuclease, ribosome-binding[20]	translation inhibition[20]
	yefM-yoeB	YoeB	YafM	*Escherichia coli*/chromosome[21]	endoribonuclease, ribosome-binding[22]	translation inhibition[22]
	yafNO	YafO	YafN	*Escherichia coli*/chromosome[23]	endoribonuclease, ribosome-binding[23]	translation inhibition[23]
	ygjNM	YgjN	YgjM	*Escherichia coli*/chromosome[23]	endoribonuclease, ribosome-binding[23]	translation inhibition[23]
	ygiUT (mqsRA)	YgiU (MqsR)	YgiT (MqsA)	*Escherichia coli*/chromosome[23]	endoribonuclease[23]	translation inhibition[23]
	dinJ-yafQ	YafQ	DinJ	*Escherichia coli*/chromosome[23]	endoribonuclease, ribosome-binding[23]	translation inhibition[23]
higBA	higBA	HigB	HigA	*Vibrio cholerae*/chromosome[24]	endoribonuclease, ribosome-binding[25]	translation inhibition[25]
vapBC	vapBC	VapC	VapB	*Mycobacterium smegmatis*/chromosome[26]	endoribonuclease[27]	translation inhibition[27]
ζε	ζε	ζ	ε	*Streptococcus pyogens*/plasmid[28]	phosphotransferase[29]	unknown
hipBA	hipBA	HipA	HipB	*Escherichia coli*/chromosome[30]	Ser/Thr kinase (target: EF-Tu)[31]	translation inhibition[32]
hicAB	hicAB (yncN/ydcQ)	HicA (YncN)	HicB (YdcQ)	*Escherichia coli*/chromosome[33]	endoribonuclease[34]	translation inhibition[34]

[1](Ogura & Hiraga, 1983); [2](Miki *et al*., 1992); [3](Saurugger, 1986); [4](Jiang *et al*., 2002); [5](Lehnherr *et al*., 1993; Magnuson & Yarmolinsky, 1998; Gazit & Sauer, 1999); [6](Liu *et al*., 2008); [7](Masuda *et al*., 1993); [8](Munoz-Gomez AJ *et al*., 2004); [9](Bravo *et al*., 1987; Bravo *et al*., 1988); [10](Zhang *et al*., 2003); [11](Tsuchimoto *et al*., 1988); [12](Zhang *et al*., 2004); [13](Masuda *et al*., 1993); [14](Zhang *et al*., 2005); [15](Zhu *et al*., 2006); [16](Fu *et al*., 2007; 2009; Zhu *et al*., 2009); [17](Lowder *et al*., 2009); [18](Bukowski *et al*., 2010); [19](Lavalle, 1965; Diderichsen *et al*., 1977; Bech *et al*., 1985; Mosteller, 1978); [20](Galvani *et al*., 2001; Pedersen *et al*., 2003); [21](Christensen *et al*., 2004); [22](Christensen-Dalsgaard & Gerdes, 2008; Zhang & Inouye, 2009); [23](Yamaguchi *et al*., 2009; Christensen-Dalsgaard *et al*., 2010); [24](Budde *et al*., 2007); [25](Christensen-Dalsgaard & Gerdes, 2006); [26](Arcus *et al*., 2005); [27](Daines *et al*., 2007; Robson *et al*., 2009); [28](Camacho *et al*., 2002; Lioy *et al*., 2006); [29](Meinhart *et al*., 2003); [30](Black *et al*., 1991; 1994; Korch & Hill, 2006); [31](Correia *et al*., 2006); [32](Schumacher *et al*., 2009); [33](Makarova *et al*., 2006); [34](Jorgensen *et al*., 2009).

hicAB and *ygiUT* systems. Binding of toxin-antitoxin complexes to promoter sites is the most common way of direct transcription regulation of TA operons (Fig. 2). Single components also bind the promoters but with a low affinity (Kedzierska *et al.*, 2007; Li *et al.*, 2008) when compared to the toxin-antitoxin oligomers which bind to palindromic sequences within the promoters, which process is enhanced cooperatively (Tsuchimoto & Ohtsubo, 1993; Black *et al.*, 1994; Magnuson *et al.*, 1996; Magnuson & Yarmolinsky, 1998; Marianovsky *et al.*, 2001; Bailey & Hayes, 2009). Moreover, apart form the described primary palindromes, promoter of the *mazEF* operon contains alternate palindromes.

Binding to the latter by a toxin-antitoxin complex manifests in a decrease in the transcription efficiency of the operon (Marianovsky *et al.*, 2001). An exception to the above rule is the prophage P1 zeta-epsilon system ($\zeta\varepsilon$) where the antitoxin serves only as an inhibitor of toxin activity and an additional expression regulator ω is present (de la Hoz *et al.*, 2000), which is similar to recently reported three-component systems homologous to *parDE*, namely *paaR1–paaA1–parE1* and *paaR2–paaA2–parE2* (Hallez *et al.*, 2010). Such a way of controlling the cellular levels of TA system components combined with high proteolysis susceptibility of the antitoxin provides the way of tight and environmentally switchable regulation. The instability of the antitoxin in a TA system is a crucial step in the system activation. It is suggested that disordered C-terminal regions of the antitoxin are target for ATP-dependent serine proteases (Kamada *et al.*, 2003). These members of chaperone family are responsible for degradation of misfolded proteins as well as components of signalling pathways (Gottesman, 1996). However, the antitoxin YgiT (MqsA) of the *ygiUT (mqsRA)* system is structured throughout its entire sequence, both free and toxin-bound state (Brown *et al.*, 2009). The activity of ATP-dependent proteases stays in a specific relation with the activity of TA systems. In all documented cases only a single protease is responsible for degradation of a particular antitoxin (although the proteases of interest comprise a family of related enzymes) (Van Melderen *et al.*, 1994; Lehnherr & Yarmolinsky, 1995; Aizenman *et al.*, 1996; Christensen *et al.*, 2001; 2004; Kawano *et al.*, 2007; Christensen-Dalsgaard *et al.*, 2010; Donegan *et al.*, 2010). Degradation of the antitoxin component leads to subsequent toxin activation and increase in operon transcription in response to a toxin and antitoxin level imbalance. However, a halt of translation, induced for example by antibiotics, acts as another way of toxin activation by causing a drop in the production of labile antitoxin.

The significant influence of the TA systems on bacterial metabolism implies multiple ways of their activity

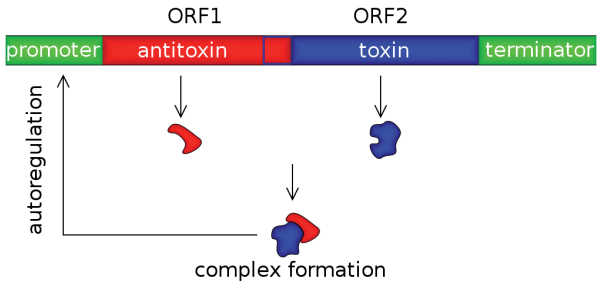

Figure 2. Binding of toxin-antitoxin complex to regulatory sequences leads to autorepression of TA operon expression

regulation. A well documented mechanism is the relation between the *mazEF* system of *E. coli* and locus *relA*, which codes for ATP:GTP 3'-diphosphotransferase implicated in the synthesis of 3',5'-guanosine bisphosphate (Justesen *et al.*, 1986; Metzger *et al.*, 1988). The ppGpp molecule is a signal of amino-acid starvation (Cashel, 1975; Gallant *et al.*, 1976). The *mazEF* locus is located downstream the *relA* locus (Masuda *et al.*, 1993) and is cotranscribed when *relA* expression is activated (Aizenman *et al.*, 1996; Christensen *et al.*, 2003; Hazan & Engelberg-Kulka, 2004). A similar neighbourhood pattern of the *mazEF* and *parDE* systems is found in genomes of other enteric bacteria such as *Shigella* and *Salmonella* (Pandey & Gerdes, 2005). Another example is the SOS system and its relations with various TA systems of *E. coli*. In this case the activation of SOS system leads to switching on the activity of TA systems including *hokE* (Fernandez De Henestrosa *et al.*, 2000), *yafNO* (McKenzie *et al.*, 2003; Christensen-Dalsgaard *et al.*, 2010), *tisAB* (Vogel *et al.*, 2004; Unoson & Wagner, 2008), *symER* (Kawano *et al.*, 2007), and *yefQ* (Motiejunaite *et al.*, 2007). A similar situation was recently reported for another *E. coli* TA system — *yafNO* (Singletary *et al.*, 2009).

The activity of TA systems can also be induced by systems responsible for *quorum sensing*. Such a mechanism has been reported for the *mazEF* system of *E. coli* (Kolodkin-Gal *et al.*, 2007). Another noteworthy fact is the possibility of cascade activation of TA systems (Hazan *et al.*, 2001) since the bacteria often carry more than a single TA system within their genome. Activation of a single system which leads to protein synthesis inhibition and subsequent activation of another TA system is plausible. An even more complex relation has been described for the *ygiUT (MqsRA)* system of *E. coli*. In this case activation of the TA system is necessary for activation of toxin CspD, whose gene promoter is controlled by the ygiU/ygiT (MqsR/MqsA) complex (Brown *et al.*, 2009; Kim *et al.*, 2010). Furthermore, a cross-regulation has been observed for homologous systems present in the genome (Yang *et al.*, 2010), where toxin-antitoxin complexes of one system bind to regulatory sequences of another TA system operon.

FUNCTIONS OF CLASS II TA SYSTEMS

A plasmid maintenance function was initially assigned to several newly discovered plasmid-borne TA systems (Gerdes & Molin, 1986; Saurugger, 1986; Bravo *et al.*, 1988; Tsuchimoto *et al.*, 1988; Gerlitz *et al.*, 1990; Sobecky *et al.*, 1996). Cells that do not inherit a copy of a plasmid upon division do not survive the effect of a stable toxin after degradation of a labile antitoxin. Moreover, a role of multiple TA *loci* in stabilization of a mega-integron of *Vibrio cholerae* has been suggested (Pandey & Gerdes, 2005). There is no doubt that TA systems play a role in the phenomenon of mobile genetic element stabilization but operons of many TA systems are also located in the bacterial chromosome. Recent studies report that TA systems are mainly concerned with the regulation of bacterial metabolism rather than simple plasmid maintenance functions.

Toxin activity leads primarily to bacterial metabolic dormancy that can be abolished at initial stages (Nystrom, 1999; Pedersen *et al.*, 2002; Keren *et al.*, 2004; Gerdes *et al.*, 2005; Suzuki *et al.*, 2005; Buts *et al.*, 2005; Lewis, 2005; Inouye, 2006; Schumacher *et al.*, 2009; Fu *et al.*, 2009; Kasari *et al.*, 2010), which contrasts with ear-

lier suggestions that this activity leads to immediate cell death (Aizenman *et al.*, 1996; Hazan & Engelberg-Kulka, 2004; Engelberg-Kulka *et al.*, 2005). There are examples of such systems whose major role is to kill the cells, but this is only true in some specialized situations. A good example are formation of fruiting bodies of *Myxococcus xanthus* (Nariya & Inouye, 2008) or defence against phage infection in lactic acidic bacteria (Forde & Fitzgerald, 1999). The question whether TA system activity leading to death of selected cells in a colony is a manifestation of an altruistic or other mechanism is currently a topic of discussion (Aizenman *et al.*, 1996; Forde & Fitzgerald, 1999; Nystrom, 1999; Lioy *et al.*, 2006).

A flexible response of a bacterial cell to stress conditions seems to be the major function of most TA systems. A reversible metabolic dormancy caused by their activation allows a bacterial cell to survive detrimental conditions. This phenomenon provides clear advantages in the case of starvation (Christensen *et al.*, 2001; Jorgensen *et al.*, 2009) as well as heat, osmotic and free-radicals-induced stress (Pedersen *et al.*, 2002; Senn *et al.*, 2005). Moreover, TA systems can contribute to the formation of persistent cells during an exposure to antibiotics (Falla & Chopra, 1998; Keren *et al.*, 2004; Dorr *et al.*, 2010; Kasari *et al.*, 2010). The mechanism of described phenomenon is straightforward in the case of drugs acting as transcription (eg. rifampicin) or translation (eg. chloramphenicol, doxycyclin, spectinomycin, eritromycin) inhibitors when the decay of the labile antitoxin causes the toxin activation. Paradoxically, antibiotics that are gyrase inhibitors (quinolone antibiotics) can act in a way similar to the *ccdAB* TA system, in which the toxin is a gyrase inhibitor. In this case binding of the inhibitor to an open gyrase–DNA complex induces DNA nicks (Drlica & Zhao, 1997; Jiang *et al.*, 2002), which is followed by SOS-system activation (Little & Mount, 1982; Karoui *et al.*, 1983; Bailone *et al.*, 1985). The same mechanism is proposed for homologues of *parDE* system (Hallez *et al.*, 2010). The described sequence of events leads to increased genetic diversity of a colony and may contribute to persisters formation (Couturier *et al.*, 1998) in the same way as do quinolone antibiotics (Drlica & Zhao, 1997).

The activity of TA systems can also modulate the behaviour of a bacterial colony. An increase in the expression of genes related to cell motility and structural genes of flagella has been reported for the *ygiUT (MqsRA)* system (Gonzalez Barrios, 2006). In turn the *hipAB* system is implicated in biofilm formation providing multi drug resistance (Lewis, 2007; 2008). TA systems can modulate formation of a biofilm over time (Kim *et al.*, 2009). In line with that, a recent report indicates elevated expression of TA systems in bacterial cells building a biofilm (Mitchell *et al.*, 2010).

A precise control over pathogenesis progression has been demonstrated for mRNA interferases exhibiting sequence specificity. This specificity allows for molecular evolution of target gene sequences. The mRNA interferases of the *mazEF-mt3* and *mazEF-mt7* systems are able to specifically recognize pentanucleotide sequences. In both cases a statistically significant representation of genes implicated in pathogenesis was found among genes containing underrepresented number of the recognized sequences (Zhu *et al.*, 2008). Such genes are resistant to the interferase activity and thereby are expected to be expressed even when the TA system is activated. A similar relation was found for the *sraP* gene of *S. aureus*. This gene, coding for a protein responsible for adhesion to platelets (Siboo *et al.*, 2005), is characterized by a statistically significant overrepresentation of the sequence recognized by the mRNA interferase of the *mazEF*$_{Sa}$ TA system (Zhu *et al.*, 2009), hence its expression is suggested to be primarily turned off upon TA system activation. Additionally, the mentioned TA system may potentially be implicated in pathogenesis progression in yet another way. Downstream of the *mazEF*$_{Sa}$ locus a *sigB* locus is located (Kullik *et al.*, 1998; Gertz *et al.*, 1999; Ferreira *et al.*, 2004). The *sigB*-encoded alternative subunit σ^B of the RNA polymerase is responsible for global transcription regulation of virulence factors, comprising one of the most important staphylococcal systems of gene regulation responsible for pathogenesis (Wu *et al.*, 1996). In stress conditions the *sigB* locus is coexpressed with *mazEF*$_{Sa}$ (Senn *et al.*, 2005; Fu *et al.*, 2007; Donegan & Cheung, 2009). However, any potential functional relation demands further investigation since the elevated expression of *sigB* locus does not necessarily lead to a direct increase in the level of σ^B subunit (Senn *et al.*, 2005). Among other pathogenic strains also *Bacillus anthracis* possesses a TA system of the mazEF family, namely a *pemIK* module (Agarwal *et al.*, 2007; 2009). Recently a *pemIK* homologue located in a plasmid of an avian strains of *S. aureus* has been documented (Lowder *et al.*, 2009; Bukowski *et al.*, 2010). In this system the toxin is a sequence-specific endoribonuclease which targets a tetranucleotide sequence. Bioinformatic analysis of the occurrence of the recognized sequence in the coding sequences of the *S. aureus* genome elucidated a potential relation of the system with virulence factor regulation (Bukowski *et al.*, 2010).

CLASS II TA SYSTEMS AS BIOTECHNOLOGICAL TOOLS

Two of the best-described TA systems have found application in molecular biology, namely *ccdAB* and *mazEF*. The former is used as a factor for positive selection of transformants, primarily in *E. coli* strains (Bernard *et al.*, 1994). Such systems, which are commercially available (e.g. StabyCloning™ and StabyExpress™, Delphi Genetics SA), are based on CcdB toxicity against gyrase and allow one-step selection of transformants ensuring stable vector plasmid maintenance (Fig. 3). This idea was originally developed by Szpirer and Milinkovitch (2005) followed by other efforts to develop a more complex system allowing increased production of recombinant protein (Stieber *et al.*, 2008).

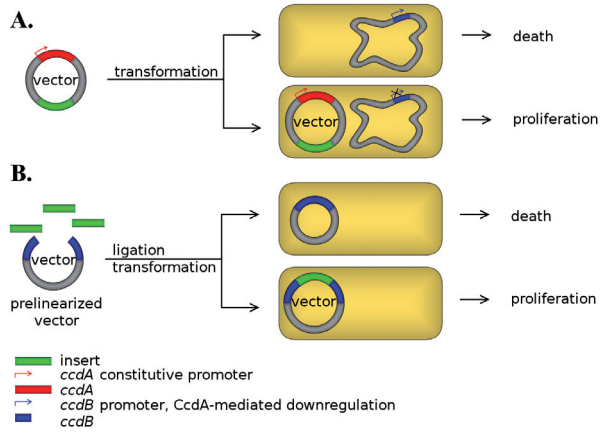

Figure 3. ccdAB system components as tools for positive selection during cloning

The *mazEF* system has been adapted for single protein production (SPP) systems. The initial idea uses MazF toxin to trigger bacteriostasis and bacterial protein shutdown. The recombinant gene lacks the ACA sequences, recognized by the MazF interferase, therefore upon induction of MazF expression production of the recombinant protein of interest is continued almost exclusively. Moreover, bacteriostasis allows for culturing of the transformed strains in lower medium volumes than in traditional methods (Suzuki *et al.*, 2005; 2007). This idea has been successfully applied for protein production for NMR studies in 150-fold concentrated cultures, which allowed significant cost saving on isotopes (Mao *et al.*, 2009; Schneider *et al.*, 2009). Recently the SPP system based on MazF activity was extended with the capability for induction of protein production using particular amino acids. MazF mutants with histidine or tryptophan substitution were used in histidine or tryptophan auxotrophs, respectively. After transferring cells to the medium enriched in isotopes but lacking one of these amino acids the production of MazF is still provided. Subsequent addition of the amino acid induces exclusive production of the recombinant protein, since production of host proteins is blocked by the toxic action of MazF. Therefore, this approach allows not only single protein production but also high-efficiency isotope-labelling of the target protein (Vaiphei *et al.*, 2010).

TA systems are successfully used also in studies on eukaryotic cells. Recently a report concerning the usage of *mazEF* system in studies on HIV virus was published (Chono *et al.*, 2010). Further possible applications have already been suggested, such as TA-based contamination control in fermentation processes (Kristoffersen *et al.*, 2000), antibacterial drug development (Engelberg-Kulka *et al.*, 2004; Moritz & Hergenrother, 2007; Lioy *et al.*, 2010), selectable elimination of cells in cell cultures, tissue cultures and whole organisms (de la Cueva-Mendez *et al.*, 2003) or stable plasmid maintenance without antibiotic pressure (Wladyka *et al.*, 2010).

CONCLUDING REMARKS

Results collected so far give a complex but concise image of the role of TA systems in bacterial physiology. Their functions range far beyond stabilization of mobile genetic elements. Metabolic dormancy induced by the systems seems a general but adequate response to various stress stimuli coming from the environment. Endoribonucleases, also termed mRNA interferases, are the most common group among the toxic components of various TA systems. Their activity leads to bacteriostasis through the inhibition of translation, which enables survival during starvation or antibiotic exposition. Further specialisation of interferases in selective sequence recognition allowed some genes to escape from expression suppression or, conversely, become exceptionally sensitive to a particular TA system. These phenomena are suggested to play a significant role in pathogen–host interaction and pathogenesis progression by modulation of biofilm formation and interactions with host proteins or coupling with other pathogen invasion-facilitating systems.

The relations among the ten families of class II TA systems are difficult to untangle. These TA systems are spread throughout the two huge domains of *Archaea* and *Bacteria*. Beside clear relationships, it seems that the similar way of acting and regulation of various groups of TA systems are due to convergence. Components of such systems could have evolved divergently from unrelated groups of genes to create autoregulated operons coding for pairs of toxic protein and its inhibitor.

The physiological functions of the TA systems became a base for their successful applications as molecular biology tools, both in industry and research. Primarily they facilitate maintenance of plasmid vectors and transformant selection, but also effective overexpression of recombinant proteins. The potential application of TA systems in antibiotic therapy cannot be omitted as it is known that TA systems induce bacteriostasis, whose prolongation results in bacterial cell death. With the growing knowledge of TA systems new useful applications are expected to be developed.

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